AD_____

Award Number: DAMD17-03-1-0567

TITLE: Therapeutic Potential of a Novel Smac/DIABLO in Breast Cancer

PRINCIPAL INVESTIGATOR: Rakesh K. Srivastava, Ph.D.

CONTRACTING ORGANIZATION: University of Maryland at Baltimore
Baltimore, Maryland 21201

REPORT DATE: February 2005

TYPE OF REPORT: Final

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	February 2005	Final (21 Jul 2003 - 20 Jan 2005)

4. TITLE AND SUBTITLE
Therapeutic Potential of a Novel Smac/DIABLO in Breast Cancer

5. FUNDING NUMBERS
DAMD17-03-1-0567

6. AUTHOR(S)
Rakesh K. Srivastava, Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
University of Maryland at Baltimore
Baltimore, Maryland 21201

E-Mail: rsrivast@rx.umaryland.edu

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT (Maximum 200 Words)

The objectives of this study was to examine the intracellular mechanisms by which Smac/DIABLO sensitizes TRAIL-resistant breast cancer cells to undergo apoptosis, and enhances the therapeutic potential of tamoxifen, doxorubicin and paclitaxel. Overexpression of Smac/DIABLO gene or Smac peptide enhances the apoptosis-inducing potential of tamoxifen, doxorubicin and paclitaxel, and sentitizes TRAIL-resistant breast cancer cell lines. The effects of combination treatments with Smac/DIABLO and drugs on apoptosis, growth inhibition, and caspase activation were higher than the single treatment alone. Thus, the combination of Smac/DIABLO with tamoxifen, doxorubicin, paclitaxel or TRAIL may be used as a new therapeutic approach for breast cancer therapy.

14. SUBJECT TERMS
TRAIL, breast cancer, Smac/DIABLO, chemotherapy, doxorubicin

15. NUMBER OF PAGES
18

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

Table of Contents

INTRODUCTION

Most anticancer agents eradicate tumor cells by the induction of apoptosis. Tumor cells, in turn, have adopted various mechanisms to resist apoptosis. Natural inhibitors of apoptosis, such as Bcl-2 and IAP family members, protect the tumor cells from the apoptotic effects of various antineoplastic agents via different mechanisms. Therefore, there is a need to enhance the apoptosis-inducing potential of chemotherapeutic drugs and sensitize drug resistant cells for effective cancer therapy.

TRAIL/Apo-2L is a novel anticancer agent that has been shown to induce apoptosis in a variety of tumor cells of diverse origin both *in vitro* and *in vivo* (1-4). TRAIL can bind to five distinct death receptors of TNF receptor family namely TRAIL-R1/DR4 , TRAIL-R2/DR5, TRAIL-R3 /DcR1/TRID, TRAIL-R4 /DcR2/TRUNDD, and osteoprotegerin (4). Binding of TRAIL to its receptors DR4 and DR5 results in recruitment of the adaptor protein FADD, which in turn recruits and activates caspase-8 (4, 5). Active caspase-8 transmits signal either by activating downstream caspase-3 or by cleaving Bid to truncated Bid (6). Translocation of tBid to mitochondria triggers Bax and Bak oligomerization, releases mitochondrial proteins, activates downstream caspases and induces apoptosis (7, 8). Caspase-8-mediated Bid processing therefore bridges the extrinsic death receptor-mediated pathway of apoptosis to the intrinsic mitochondrial pathway (8, 9). This provides a mechanism to amplify the execution signal and exacerbate the pace of cell demise. The Bcl-2 family proteins regulate apoptosis by acting mainly at the level of mitochondria (10).

Mitochondrial membrane potential plays an important role in the exchange of ions and various molecules for the formation of apoptosomes (11, 12). Loss of membrane potential leads to opening of the permeability transition pore leaking the inner components into cytosol, which provide the executing signals for apoptosis (13, 14). The release of mitochondrial protein cytochrome c is essential for the formation of apoptosomes and activation of caspase-9 in type II cells (6, 15, 16). Activation of caspase-9 causes activation of down-stream caspases (caspase-3, caspase-6, and caspase-7), which result in apoptosis. Like cytochrome c, Smac/DIABLO is localized in mitochondria (17). Based largely upon *in vitro* studies, Smac/DIABLO appears to

function by neutralizing the caspase-inhibitory properties of the IAP family of proteins by binding to BIR2 and BIR3 domains, particularly XIAP (18-20). XIAP is an endogenous inhibitor of caspase-3, -7, and -9. Current data suggest a model whereby the ability of XIAP to repress active caspase-9 within the apoptosome complex is overcome by displacement of XIAP from caspase-9 by Smac/DIABLO (21, 22). Similarly, HtrA2/Omi is released from mitochondria and inhibits the function of XIAP by direct binding in a similar way to Smac (23, 24).

Smac/DIABLO is encoded by a nuclear gene and is subsequently imported into mitochondria (17, 24). The N-terminus of Smac (55 residues containing the mitochondrial targeting sequences, MTS) is removed by proteolysis to generate the mature and functional form (containing 184 amino acids) of the molecule during mitochondrial import (17, 22, 24). Interestingly, mature Smac/DIABLO exists as a dimer, mediated by a hydrophobic interface within the N-termini of individual Smac/DIABLO molecules (22). Mutations that disrupt Smac/DIABLO dimer formation abrogate the XIAP-neutralizing ability of this molecule, suggesting that Smac/DIABLO dimerization is essential for its proapoptotic activity (22). Ectopic overexpression of Smac potentiates epothilone B derivative (BMS)-induced apoptosis (25). Furthermore, Smac agonists sensitized various tumor cells *in vitro* and malignant glioma cells *in vivo* for apoptosis induced by death-receptor ligation or cytotoxic drugs (26). Most importantly, Smac peptides strongly enhanced the antitumor activity of TRAIL in an intracranial malignant glioma xenograft model *in vivo* (26). Complete eradication of established tumors and survival of mice was only achieved upon combined treatment with Smac peptides and TRAIL without detectable toxicity to normal brain tissue (26). Thus, Smac agonists are promising candidates for cancer therapy by potentiating cytotoxic therapies.

Hypothesis. We hypothesize that Smac/DIABLO enhances the apoptosis-inducing potential of chemotherapeutic drugs and sensitizes TRAIL-resistant cells by promoting caspase-3 activity and by neutralizing the inhibitory effect of IAPs.

Objective: The objective of the project was to investigate the clinical potential of the second mitochondria-derived activator of caspase (Smac/DIABLO) in enhancing the apoptosis-inducing potential of commonly used anticancer drugs (paclitaxel, doxorubicin, and tamoxifen) and TRAIL in breast carcinoma.

BODY

Interactive effects of Smac/DIABLO with chemotherapeutic drugs or TRAIL on cell viability and apoptosis

We have taken two approaches to examine the effects of Smac/DIABLO in breast cancer cells. In first approach, the Smac/DIABLO (Smac/DIABLO N7, H-AVPIAQK-OH) and control peptides were used. In the second approach, cells were transfected with plasmids expressing full length Smac/DIABLO (pCDNA3-Smac/DIABLO-flag), Δ55 Smac/DIABLO (pCDNA3- Δ55 Smac/DIABLO-flag) or neo (pCDNA3-neo-flag). The NH2 terminus of Smac/DIABLO (55 residues containing the MTSs) is removed by proteolysis to generate the mature and functional form (containing 184 amino acid) of the molecule during mitochondrial import. The goal was to increase the amount of Smac in the cytosol either by pharmacological or genetic method.

We first measured the effects of Smac/DIABLO on cell viability and apoptosis of MCF-7 breast cancer cells (Fig. 1). Control Smac/DIABLO peptide (Smac/DIABLO C) had no effect on cell viability and apoptosis. Smac/DIABLO peptide (Smac/DIABLO P) slightly inhibited viability and induced apoptosis (Fig. 1 A and B). Treatment of MCF-7 cells with Smac/DIABLO peptide increased the effects of TRAIL on cell viability and apoptosis. Overexpression of full length Smac/DIABLO or Δ55 Smac/DIABLO enhanced TRAIL-induced apoptosis in MCF-7 cells (Fig. 1C). We next examined the effects of Smac/DIABLO on TRAIL-resistant MDA-MB-453 and MDA-MB-468 breast cancer cells. Smac/DIABLO peptide (25 -100 μM) sensitized TRAIL-resistant MDA-MB-

453 and MDA-MB-468 breast cancer cells in a dose-dependent manner (Fig. 1 D and E).

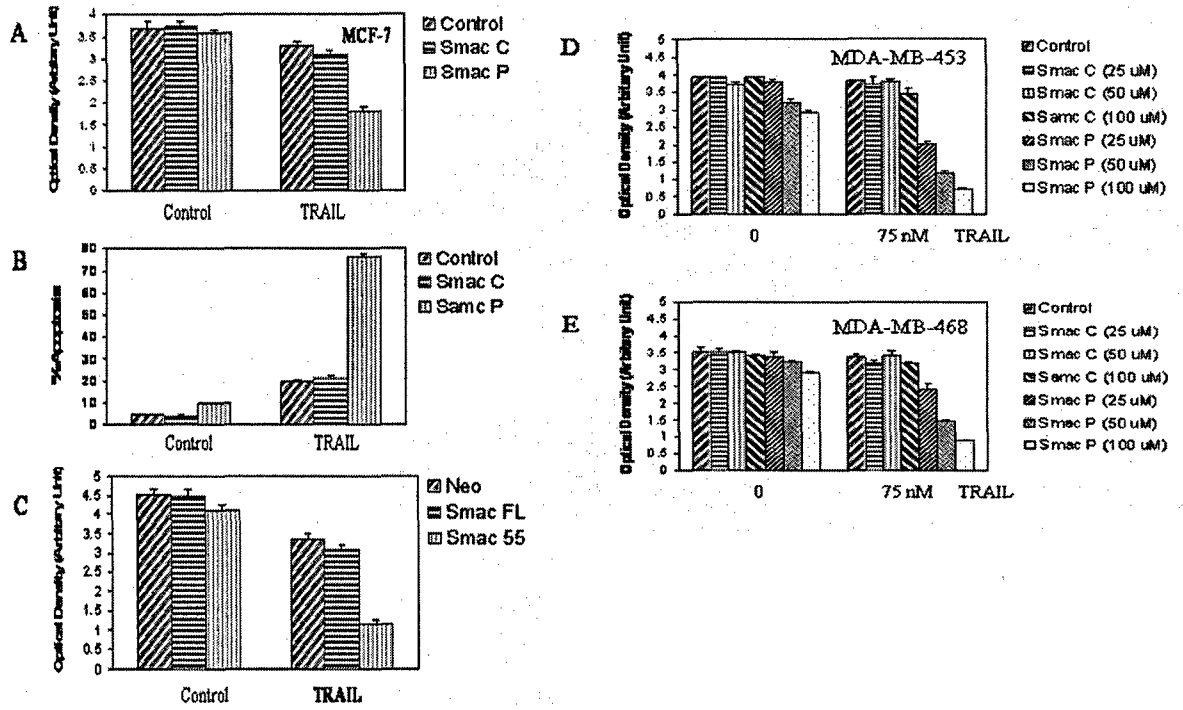
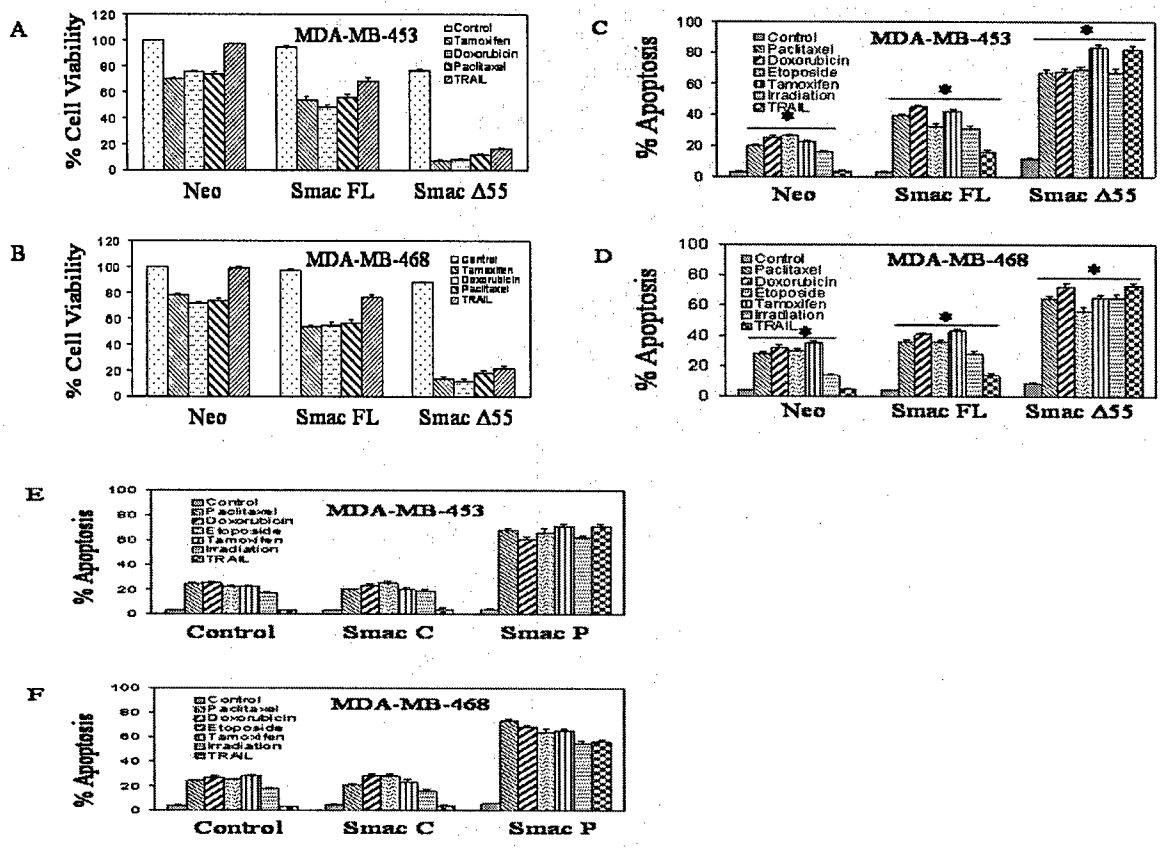


Fig. 1. Interactive effects of Smac/DIABLO with TRAIL on cell viability and apoptosis. (A), MCF-7 cells were pretreated with either Smac/DIABLO control peptide (25 μM) or Smac/DIABLO N7 peptide (25 μM) for 12 h, followed by treatment with TRAIL (75 nM) for 36 h. Cell viability was measured by XTT assay. (B), MCF-7 cells were pretreated with either Smac/DIABLO control peptide (25 μM) or Smac/DIABLO N-7 peptide (25 μM) for 12 h, followed by treatment with TRAIL (75 nM) for 36 h. Apoptosis was measured by Annexin V-FITC and P.I. staining. (C), MCF-7 cells were transiently transfected with plasmids expressing Smac/DIABLO full length, Smac Δ55 or neo for 24 h, and treated with TRAIL (75 nM) for 36 h. Cell viability was measured by XTT assay. Data represent mean ± SE. Samc C = Samc control peptide, Smac P = Smac N7 peptide. (D), MDA-MB-453 cells were pretreated with various doses of either Smac/DIABLO control peptide (0-100 μM) or Smac/DIABLO N-7 peptide (0-100 μM) for 12 h, followed by treatment with TRAIL (75 nM) for 36 h. Cell viability was measured by XTT assay. Data represent mean ± SE. (E), MDA-MB-468 cells were pretreated with

various doses of either Smac/DIABLO control peptide (0-100 μM) or Smac/DIABLO N-7 peptide (0-100 μM) for 12 h, followed by treatment with TRAIL (75 nM) for 36 h. Cell viability was measured by XTT assay. Data represent mean ± SE.

In addition to TRAIL, we have also used commonly used anticancer drugs (tamoxifen, doxorubicin and paclitaxel) and irradiation as therapeutic agents. Overexpression of full-length Smac/DIABLO (Smac/DIABLO FL) or mature form of Smac/DIABLO (Smac/DIABLO Δ55) enhanced the inhibitory effects of tamoxifen, doxorubicin, and paclitaxel on cell viability, and sensitized MDA-MB-453 and MDA-MB-468 cells to TRAIL (Fig. 2A and B). Furthermore, overexpression of Smac/DIABLO FL or Smac/DIABLO Δ55 enhanced paclitaxel-, doxorubicin-, etoposide, tamoxifen-, and irradiation-induced apoptosis, and sensitized MDA-MB-453 and MDA-MB-468 cells to TRAIL (Fig. 2C and D). Smac/DIABLO Δ55 was more effective in inhibiting cell viability and promoting apoptosis compared to full length Smac/DIABLO.



Since overexpression of Smac/DIABLO enhanced the apoptosis-inducing potential of chemotherapeutic drugs and irradiation, and sensitize TRAIL-resistant cells, we next sought to examine the interactive effects of Smac/DIABLO peptide with these agents (Fig. 2 E and F). As expected, Smac/DIABLO peptide enhanced paclitaxel-, doxorubicin-, etoposide-, tamoxifen-, and irradiation-induced apoptosis, and sensitized TRAIL-resistant MDA-MB-453 and MDA-MB-468 cells to TRAIL. Control peptide, in the presence or absence of drugs or irradiation, had no effect on apoptosis.

Fig. 2. Effects of Smac/DIABLO with chemotherapeutic drugs or TRAIL on cell viability. (A), MDA-MB-453 cells were transiently transfected with plasmids expressing Smac/DIABLO full length, Smac Δ55 or neo for 24 h, and treated with tamoxifen (100 nm), doxorubicin (100 nM), paclitaxel (100 nM) or TRAIL (75 nM) for 36 h. Cell viability was measured by XTT assay. Data represent mean ± SE. (B), MDA-MB-468 cells were transiently transfected with plasmids expressing Smac/DIABLO full length, Smac Δ55 or neo for 24 h, and treated with tamoxifen (100 nM), doxorubicin (100 nM), paclitaxel (100 nM) or TRAIL (75 nM) for 36 h. Cell viability was measured by XTT assay. Data represent mean ± SE. (C), MDA-MB-453 cells were transiently transfected with plasmids expressing Smac/DIABLO full length, Smac Δ55 or neo for 24 h, and treated with paclitaxel (100 nM), doxorubicin (100 nM), etoposide (100 nM), tamoxifen (100 nm), irradiation (5 Gy) or TRAIL (75 nM) for 36 h. Apoptosis was measured by DAPI staining. Data represent mean ± SE. (D), MDA-MB-468 cells were transiently transfected with plasmids expressing Smac/DIABLO full length, Smac Δ55 or neo for 24 h, and treated with paclitaxel (100 nM), doxorubicin (100 nM), etoposide (100 nM), tamoxifen (100 nm), irradiation (5 Gy) or TRAIL (75 nM) for 36 h. Apoptosis was measured by DAPI staining. Data represent mean ± SE. (E), MDA-MB-453 cells were pretreated with either Smac control peptide (25 μM) or Smac N-7 peptide (25 μM) for 12 h, and treated with paclitaxel (100 nM), doxorubicin (100 nM), etoposide (100 nM), tamoxifen (100 nm), irradiation (5 Gy) or TRAIL (75 nM) for 36 h. Apoptosis was measured by DAPI staining. Data represent mean ± SE. (F), MDA-MB-468 cells were pretreated with either Smac control peptide (25 μM) or Smac N-7 peptide (25 μM) for 12

h, and treated with paclitaxel (100 nM), doxorubicin (100 nM), etoposide (100 nM), tamoxifen (100 nm), irradiation (5 Gy) or TRAIL (75 nM) for 36 h.

Interactive effects of Smac/DIABLO with chemotherapeutic drugs or TRAIL on colony formation

Since Smac/DIABLO peptide enhanced the apoptosis-inducing potential of anticancer drugs, and sensitized TRAIL resistant breast cancer cells, we sought to examine whether they have similar effects on colony formation (Fig. 3). Tamoxifen, doxorubicin and paclitaxel inhibited colony formation in both MDA-MD-453 and MDA-MB-468 cells. In contrast, TRAIL or control peptide had no effect on colony formation. Similar to apoptosis, Smac/DIABLO peptide enhanced the inhibitory effects of anticancer drugs on colony formation, and sensitized TRAIL-resistant cells.

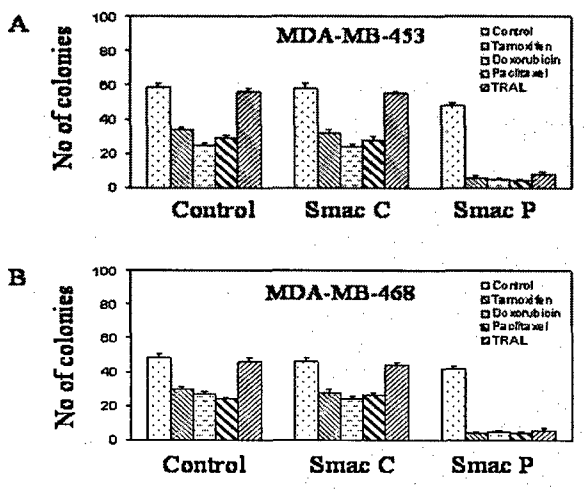


Fig. 3. Interactive effects of Smac/DIABLO peptide with chemotherapeutic drugs or TRAIL on colony formation. (A), MDA-MB-453 cells were pretreated with either Smac control peptide (25 μM) or Smac N-7 peptide (25 μM) for 12 h, and treated with tamoxifen (100 nm), doxorubicin (100 nM), paclitaxel (100 nM) or TRAIL (75 nM) for 5 days. No. of colonies were determined by soft agar assay. Data represent mean ± SE. (B), MDA-MB-468 cells were pretreated with either Smac control peptide (25 μM) or Smac N-7 peptide (25 μM) for 12 h, and treated with tamoxifen (100 nm), doxorubicin (100 nM), paclitaxel (100 nM) or TRAIL (75 nM) for 5 days. No. of colonies were determined by soft agar assay. Data represent mean ± SE.

Smac/DIABLO enhances drug-induced apoptosis and sensitizes TRAIL-resistant cells through caspase-3 activation

Since Smac/DIABLO augment drug-induced apoptosis and sensitizes TRAIL-resistant cells, we sought to examine the mechanism of this interaction by measuring caspase-3 activation. Tamoxifen, doxorubicin and paclitaxel induced caspase-3 activity in both MDA-MD-453 and MDA-MB-468 cells. In contrast, TRAIL or control peptide alone had no effect on caspase-3 activity (Fig. 4 A and B). Treatment of cells with Smac/DIABLO peptide further enhanced drug-induced caspase-3 activation. Furthermore, the combination of Smac/DIABLO and TRAIL resulted in caspase-3 activity in TRAIL-resistant breast cancer cells. Similar effects were obtained with full length Smac/DIABLO (Smac/DIABLO FL) and Smac/DIABLO Δ55, although Smac/DIABLO Δ55 was more potent than Smac/DIABLO FL (Fig. 4 C and D). These data suggest that Smac/DIABLO enhances the apoptosis-inducing potential of anticancer drugs and sensitizes TRAIL-resistant cells through caspase-3 activation.

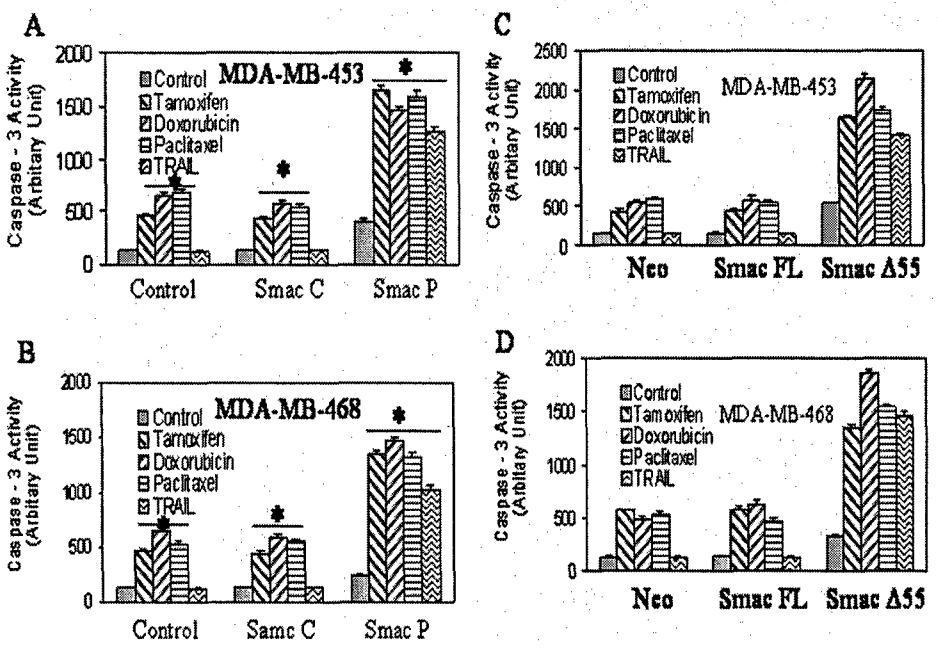


Fig. 4. Interactive effects of Smac/DIABLO peptide with chemotherapeutic drugs or TRAIL on caspase-3 activity. (A), MDA-MB-453 cells were pretreated with either Smac control peptide (25 μM) or Smac N-7 peptide (25 μM) for 12 h, and treated with tamoxifen (100 nm), doxorubicin (100 nM), paclitaxel (100 nM) or TRAIL (75 nM) for 24

h. Caspase-3 activity was measured as per the manufacturer's instructions (Calbiochem). Data represent mean ± SE. (B), MDA-MB-468 cells were pretreated with either Smac control peptide (25 μM) or Smac N-7 peptide (25 μM) for 12 h, and treated with tamoxifen (100 nm), doxorubicin (100 nM), paclitaxel (100 nM) or TRAIL (75 nM) for 24 h. Caspase-3 activity was measured as per the manufacturer's instructions (Calbiochem). Data represent mean ± SE. (C), MDA-MB-453 cells were transiently transfected with plasmids expressing Smac/DIABLO full length, Smac Δ55 or neo for 24 h, and treated with tamoxifen (100 nm), doxorubicin (100 nM), paclitaxel (100 nM) or TRAIL (75 nM) for 24 h. Caspase-3 activity was measured as per the manufacturer's instructions (Calbiochem). Data represent mean ± SE. (D), MDA-MB-468 cells were transiently transfected with plasmids expressing Smac/DIABLO full length, Smac Δ55 or neo for 24 h, and treated with tamoxifen (100 nm), doxorubicin (100 nM), paclitaxel (100 nM) or TRAIL (75 nM) for 24 h. Caspase-3 activity was measured as per the manufacturer's instructions (Calbiochem).

Effects of Chemotherapeutic drugs on PARP cleavage

Activation of caspase results in cleavage of several substrates such as poly ADP ribose polymerase (PARP) enzyme that can be used to confirm apoptosis (6, 27). MDA-MB-468 cells were transiently transfected with plasmids expressing Smac/DIABLO (MDA-MB-468/Smac/DIABLO) or neo (MDA-MB-468/neo), and treated with or without doxorubicin, TRAIL, tamoxifen or paclitaxel, for 48 h, and cleavage of PARP was determined by the Western blot analysis (Fig. 5). The antibody recognizes only the cleavage product of PARP. Chemotherapeutic drugs significantly induced PARP cleavage. Overexpression of Smac/DIABLO resulted in enhanced cleavage of PARP in MDA-MB-468/Smac/DIABLO cells treated with doxorubicin, TRAIL, tamoxifen or paclitaxel.

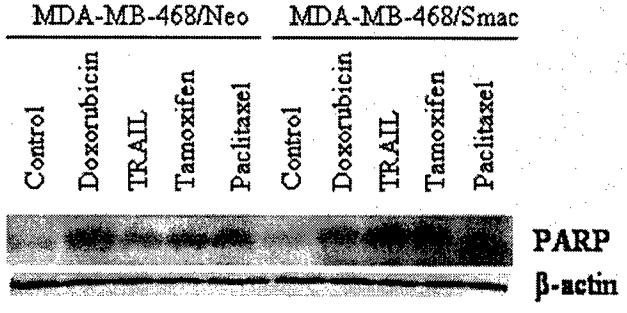


Fig. 5. Interactive effects of Smac/DIABLO with chemotherapeutic drugs or TRAIL on PARP cleavage. MDA-

MB-468 cells were transiently transfected with plasmids expressing Smac/DIABLO full length or neo for 24 h, and treated with doxorubicin (100 nM), TRAIL (75 nM), tamoxifen (100 nm) or paclitaxel (100 nM) for 24 h. Western blot analysis was performed to measure the cleavage of PARP. Anti-β-actin antibody was used as a loading control.

Interaction of Smac/DIABLO with IAPs

The apoptotic death of cells requires proteolytic activation of caspases which are synthesized as latent proenzymes (28, 29). Once activated, caspases cleave a wide range of molecules (e.g. PARP) that eventually result in the dismantlement of cells (2, 30). Active caspases can be specifically inhibited by inhibitors of apoptosis (IAP). IAP antagonists (Smac/DIABLO, Omi/HtrA2 and GSPT1/eRF3) compete with caspases for IAP-binding and consequently relieve caspases and promote cell death. Since Smac/DIABLO augments drug-induced apoptosis, and sensitizes TRAIL-resistant cells, we sought to examine the interactions of Smac/DIABLO with cIAP1, cIAP2 and XIAP. MDA-MB-468/Neo and MDA-MB-468/Smac/DIABLO cells were treated with doxorubicin, paclitaxel, tamaxifen or TRAIL for 24 h (Fig. 6). Cell lysates were immunoprecipitated with anti-Smac/DIABLO antibody, and immunoblotted with anti-cIAP1, cIAP2 or XIAP antibodies. Treatment of MDA-MB-468/neo with chemotherapeutic drugs enhanced the interaction of Smac/DIABLO with IAPs. Interactions of Smac/DIABLO and IAPs were further increased when cells were transfected with Smac/DIABLO. These data suggest that the ability of Smac/DIABLO to enhance drug-induced apoptosis is due to sequestration of IAPs, which, in turn, causes caspase activation and apoptosis.

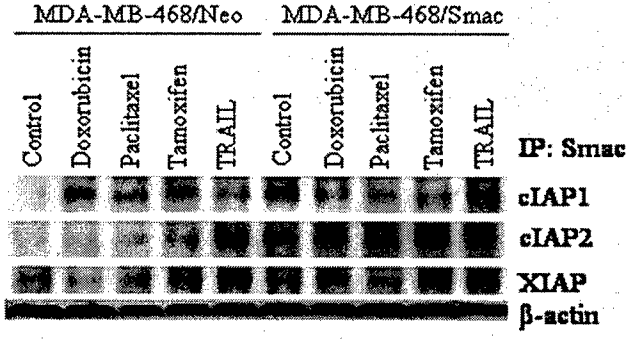


Fig. 6. Interaction of Smac/DIABLO with IAPs. MDA-MB-468 cells were transiently transfected with plasmids expressing Smac/DIABLO full length (MDA-

MB-468/Smac) or neo (MDA-MB-468/neo) gene for 24 h, and treated with doxorubicin (100 nM), paclitaxel (100 nM), tamoxifen (100 nm) or TRAIL (75 nM) for 24 h. Cells were harvested, and immunoprecipitated with anti-Smac antibody. Immunoprecipitated complexes were subjected to SDS-PAGE and immunoblotted with anti-cIAP1, anti-cIAP2 or anti-XIAP antibody. Anti-β-acting antibody was used as a control in whole cell lysate.

KEY RESEARCH ACCOMPLISHMENTS

- Overexpression of Smac/DIABLO gene (full length or Δ55) or treatment with Smac/DIABLO peptide enhanced apoptosis induced by paclitaxel, doxorubicin, and tamoxifen in breast cancer cells.
- Exogenous Smac/DIABLO resulted in an increased interaction of Smac with IAPs, which correlated with increase caspase-3 activity and apoptosis.
- Smac/DIABLO (gene and peptide) sensitized TRAIL-resistant breast cancer cell lines to undergo apoptosis through caspase-3 activation. These data suggest that apoptotic events down-stream of mitochondria were intact in TRAIL-resistant cells since ectopic expression of Smac/DIABLO or pretreatment of cells with Smac peptide completely restored TRAIL sensitivity.
- Treatment of nude mice bearing MDA-MB-468 xenografts with Smac/DIABLO gene or Smac peptide enhanced the effects of chemotherapeutic drugs (paclitaxel, tamoxifen and doxorubicin) and sensitized TRAIL-resistant cells to undergo apoptosis.
- The ability of Smac/DIABLO agonists to sensitize TRAIL-resistant cells to undergo apoptosis suggests that Smac/DIABLO may induce fundamental alterations in cell signaling pathways. Thus, Smac/DIABLO agonists can be used as promising new candidates for breast cancer treatment by potentiating cytotoxic therapies.

REPORTABLE OUTCOMES

Presentations

1. Shankar, S., M. Asim, and R.K. Srivastava. Smac/DIABLO agonists sensitize Apo2L/TRAIL- or anticancer drug-induced apoptosis in human breast, prostate and lung cancer. 94th Annual Meeting of the American Association for Cancer Research, Washington, D.C., July 11-14, 2003.
2. Shankar, S., M. Asim, and R.K. Srivastava. Therapeutic potential of Smac/DIABLO in cancer. 8th World Congress on Advances in Oncology, and 6th International Symposium on Molecular Medicine. Athens, Greece, October 16-18, 2003.
3. Singh, T.R., S. Shankar, and R. K. Srivastava. Clinical Significance of TRAIL in cancer. 8th World Congress on Advances in Oncology, and 6th International Symposium on Molecular Medicine. Athens, Greece, October 16-18, 2003.
4. Shankar, S., M. Asim, and R.K. Srivastava. Sensitization of breast cancer cells to chemotherapy and ionizing radiation by Smac/DIABLO. 95th Annual Meeting of the American Association for Cancer Research, Orlando, FL, March 27-31, 2004.
5. Shankar, S., T. Fandy, M. Asim, and R.K. Srivastava. Therapeutic potential of a novel Smac/DIABLO in breast cancer. Era of the Hope Meeting, Department of Defense US Army, Philadelphia, PA, June 8-11, 2005.

CONCLUSIONS

- Overexpression of Smac/DIABLO gene or Smac peptide enhances the apoptosis inducing potential of chemotherapeutic drugs.
- Smac/DIABLO gene or peptide sensitizes TRAIL-resistant breast cancer cells.

- Smac/DIABLO agonists are promising candidates for enhancing therapeutic potential of anticancer drugs and TRAIL for the treatment of breast cancer.

REFERENCES

1. Ashkenazi A, Pai RC, Fong S, Leung S, Lawrence DA, Marsters SA, et al. Safety and antitumor activity of recombinant soluble Apo2 ligand. J Clin Invest 1999;104(2):155-62.
2. Singh TR, Shankar S, Chen X, Asim M, Srivastava RK. Synergistic interactions of chemotherapeutic drugs and tumor necrosis factor-related apoptosis-inducing ligand/Apo-2 ligand on apoptosis and on regression of breast carcinoma in vivo. Cancer Res 2003;63(17):5390-400.
3. Shankar S, Chen X, Srivastava RK. Effects of sequential treatments with chemotherapeutic drugs followed by TRAIL on prostate cancer in vitro and in vivo. Prostate 2005;62(2):165-86.
4. Shankar S, Srivastava RK. Enhancement of therapeutic potential of TRAIL by cancer chemotherapy and irradiation: mechanisms and clinical implications. Drug Resist Updat 2004;7(2):139-56.
5. Walczak H, Bouchon A, Stahl H, Krammer PH. Tumor necrosis factor-related apoptosis-inducing ligand retains its apoptosis-inducing capacity on Bcl-2- or Bcl-xL-overexpressing chemotherapy-resistant tumor cells. Cancer Res 2000;60(11):3051-7.
6. Suliman A, Lam A, Datta R, Srivastava RK. Intracellular mechanisms of TRAIL: apoptosis through mitochondrial- dependent and -independent pathways. Oncogene 2001;20(17):2122-33.
7. Wei MC, Zong WX, Cheng EH, Lindsten T, Panoutsakopoulou V, Ross AJ, et al. Proapoptotic BAX and BAK: a requisite gateway to mitochondrial dysfunction and death. Science 2001;292(5517):727-30.
8. Kandasamy K, Srinivasula SM, Alnemri ES, Thompson CB, Korsmeyer SJ, Bryant JL, et al. Involvement of Proapoptotic Molecules Bax and Bak in Tumor Necrosis Factor-related Apoptosis-inducing Ligand (TRAIL)-induced Mitochondrial Disruption and Apoptosis: Differential Regulation of Cytochrome c and Smac/DIABLO Release. Cancer Res 2003;63(7):1712-21.
9. Luo X, Budihardjo I, Zou H, Slaughter C, Wang X. Bid, a Bcl2 interacting protein, mediates cytochrome c release from mitochondria in response to activation of cell surface death receptors. Cell 1998;94(4):481-90.
10. Korsmeyer SJ. BCL-2 gene family and the regulation of programmed cell death. Cancer Res 1999;59(7 Suppl):1693s-1700s.
11. Green DR. Apoptotic pathways: the roads to ruin. Cell 1998;94(6):695-8.
12. Srivastava RK, Sollott SJ, Khan L, Hansford R, Lakatta EG, Longo DL. Bcl-2 and Bcl-X(L) block thapsigargin-induced nitric oxide generation, c-Jun NH(2)-terminal kinase activity, and apoptosis. Mol Cell Biol 1999;19(8):5659-74.

13. Green DR. Apoptosis. Death deceiver [news; comment]. Nature 1998;396(6712):629-30.
14. Kroemer G, Dallaporta B, Resche-Rigon M. The mitochondrial death/life regulator in apoptosis and necrosis. Annu Rev Physiol 1998;60:619-42.
15. Liu X, Kim CN, Yang J, Jemmerson R, Wang X. Induction of apoptotic program in cell-free extracts: requirement for dATP and cytochrome c. Cell 1996;86(1):147-57.
16. Li F, Srinivasan A, Wang Y, Armstrong RC, Tomaselli KJ, Fritz LC. Cell-specific induction of apoptosis by microinjection of cytochrome c. Bcl-xL has activity independent of cytochrome c release. J Biol Chem 1997;272(48):30299-305.
17. Du C, Fang M, Li Y, Li L, Wang X. Smac, a mitochondrial protein that promotes cytochrome c-dependent caspase activation by eliminating IAP inhibition. Cell 2000;102(1):33-42.
18. Roy N, Deveraux QL, Takahashi R, Salvesen GS, Reed JC. The c-IAP-1 and c-IAP-2 proteins are direct inhibitors of specific caspases. Embo J 1997;16(23):6914-25.
19. Deveraux QL, Reed JC. IAP family proteins--suppressors of apoptosis. Genes Dev 1999;13(3):239-52.
20. Deveraux QL, Roy N, Stennicke HR, Van Arsdale T, Zhou Q, Srinivasula SM, et al. IAPs block apoptotic events induced by caspase-8 and cytochrome c by direct inhibition of distinct caspases. Embo J 1998;17(8):2215-23.
21. Srinivasula SM, Hegde R, Saleh A, Datta P, Shiozaki E, Chai J, et al. A conserved XIAP-interaction motif in caspase-9 and Smac/DIABLO regulates caspase activity and apoptosis. Nature 2001;410(6824):112-6.
22. Chai J, Du C, Wu JW, Kyin S, Wang X, Shi Y. Structural and biochemical basis of apoptotic activation by Smac/DIABLO. Nature 2000;406(6798):855-62.
23. Suzuki Y, Takahashi-Niki K, Akagi T, Hashikawa T, Takahashi R. Mitochondrial protease Omi/HtrA2 enhances caspase activation through multiple pathways. Cell Death Differ 2004;11(2):208-16.
24. Verhagen AM, Silke J, Ekert PG, Pakusch M, Kaufmann H, Connolly LM, et al. HtrA2 promotes cell death through its serine protease activity and its ability to antagonize inhibitor of apoptosis proteins. J Biol Chem 2002;277(1):445-54.
25. Guo F, Nimmanapalli R, Paranawithana S, Wittman S, Griffin D, Bali P, et al. Ectopic overexpression of second mitochondria-derived activator of caspases (Smac/DIABLO) or cotreatment with N-terminus of Smac/DIABLO peptide potentiates epothilone B derivative-(BMS 247550) and Apo- 2L/TRAIL-induced apoptosis. Blood 2002;99(9):3419-26.
26. Fulda S, Wick W, Weller M, Debatin KM. Smac agonists sensitize for Apo2L/TRAIL- or anticancer drug-induced apoptosis and induce regression of malignant glioma in vivo. Nat Med 2002;8(8):808-15.
27. Kaufmann SH, Desnoyers S, Ottaviano Y, Davidson NE, Poirier GG. Specific proteolytic cleavage of poly(ADP-ribose) polymerase: an early marker of chemotherapy-induced apoptosis. Cancer Res 1993;53(17):3976-85.
28. Nicholson DW, Thornberry NA. Caspases: killer proteases. Trends Biochem Sci 1997;22(8):299-306.

29. Salvesen GS, Dixit VM. Caspase activation: the induced-proximity model. Proc Natl Acad Sci U S A 1999;96(20):10964-7.
30. Kaufmann SH, Earnshaw WC. Induction of apoptosis by cancer chemotherapy. Exp Cell Res 2000;256(1):42-9.